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Random genomic clones as a tool to construct genetic map in Japanese medaka, *Orizias latipes*

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The aquarium fish, such as zebrafish (*Brachydanio rerio*) and Japanese medaka (*Orizias latipes*), are highlighted as an excellent model for studies on vertebrate development and *in vivo* screening of suspect carcinogens (1). The benefit of fish model over mice for these researches is its small size and therefore, ease of rearing a larger number of animals more cheaply, and a large number of embryos produced from one female. The transparency of embryo and rapid development also provide advantage to detect congenital deformities derived from mutation and developmental anomalies following exposures to a certain mutagenic agent easily from a large number of embryos. Recently, many of homeotic genes homologous to mammalian and *Drosophila* system have been cloned from zebrafish genome (2, 3, 4, 5). Their spatial and developmental stage restricted expressions suggest developmental comparability among them.

Medaka has unique feature that is not seen in the other fish models. For toxicological study, non-mutagenic and formulated diet for medaka was already developed (6). Shima and Shimada (7) established a strain for evaluating radiation induced mutations. A line of these studies provides importance of medaka in the study of toxicology and mutagenicity. Additionally, it became possible to microinject genes into medaka embryos (8). A particularly important feature of medaka is the availability of inbred strains of medaka (9, 10). These strains are genetically well controlled and promise consistent results. Despite these attractions, medaka model lacks a basic tool that is taken for granted in the mouse. There is no genetic map. Apparently, increasing knowledge of mouse and human biology is based on its fertile genetic background, including fine genetic map. For constructing detailed genetic map, random genomic probe is useful, as well known in human genome project.

Sakaizumi *et al.* (11) demonstrated that wild Japanese medaka is composed of two genetically divergent populations, the Southern population and the Northern population. Genetic analysis suggested that these two populations differentiated more than one million years ago. Large genetic difference can be expected in the two populations. Hyodo-Taguchi (9, 10) established several inbred strains of medaka from the two wild populations. Therefore, it is likely to find many genetic polymorphisms in the inbred strains.

In this study, we cloned many random genomic probes from an inbred strain of medaka (HO5) to construct genetic map of two inbred strains of medaka, HO4C and HNI derived from the Southern population and the Northern population, respectively.

Medaka

Details of characters of medaka inbred strains were described elsewhere (9, 10). The HO5 and HO4C strains were established from the orange-red variety of medaka, originated from Southern population in Japan. The HNI strain was developed from wild medaka collected from Niigata which belongs to the Northern population.

Cloning of random genomic probes from medaka genomic DNA

Head, intestine and fins were removed from the adult male medaka (HO5). The DNA was extracted in extraction buffer (50 mM Tris-HCl pH 8.0, 100 mM EDTA, 100 mM NaCl, 1% SDS and 35 μg/ml Protenase K) at 37°C overnight, and further purified by phenol-chlolroform extraction and ethanol precipitation. 4 μg of the extracted DNA was digested completely with 40U *EcoR*I and size-fractionated by electrophoresis. 0.5–3Kb of the digested DNA was collected and ligated with *EcoR*I site of pUC119. Ligated DNAs were used for transformation to *E.coli* DH5α. Fifty randomly

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selected clones were grown in the 5 ml Brain-Heart Infusion culture media for 16 hr. Plasmid DNAs from each clone were extracted by using a Magic minipreps (ProMega) column, and used for Southern blot analysis as probes.

Southern blot analysis

Genomic DNA of HO4C and HNI male adult medaka was extracted by the method described above. 5 μg of the DNAs was completely digested with several restriction enzymes. Digested DNAs were transferred onto Hybond-N$^+$ (Amersham) and was probed with each probe described above. Blots were hybridized in 0.3M $NaHPO_4$ (pH 7.3), 7% SDS and 1 mM EDTA at 65°C. Blots were washed three times in 0.2 X SSC and 0.1% SDS at 67°C.

Usefulness of random genomic DNA of medaka to gene mapping

Fifty independent random clones were obtained in our experiment and designated as pHO5-1 through 50. In the present study, 16 out of the 50 clones were analyzed for restriction fragment length polymorphism (RFLP) between HO4C and HNI. An example of hybridization and summarized data are shown in Figure 1 and Table 1, respectively. Southern blot analysis showed that 10 out of 16 clones gave one or two bands, whereas multiple or smear bands were observed by the other probes. RFLPs between HO4C and HNI after digestion with eight restriction enzymes (*Hin*dIII, *Pvu*II, *Sac*I, *Apa*I, *Eco*RV, *Pst*I, *Eco*RI and *Bgl*II) were observed by all 10 probes. These data suggested that two inbred strains of medaka, HO4C and HNI, have large DNA polymorphisms. Such large differences observed here have been already shown by protein polymorphism data (11). Our results suggest that random genomic clone is efficient to find the genetic difference among inbred strains of medaka. This method can be applied to construction of genetic map in medaka. Generally, gene mapping is laborous and time consuming. By using these DNA clones as anchor probes of certain chromosome, a gross genetic map in medaka could be established efficiently. After that, it is easy to expand the mapping data to functional genes and proteins.

We have just started this project extensively. About 200 DNA random clones were isolated from male HO5 genomic DNA. There is a good reason to believe that this project will make the medaka a useful laboratory animal still more.

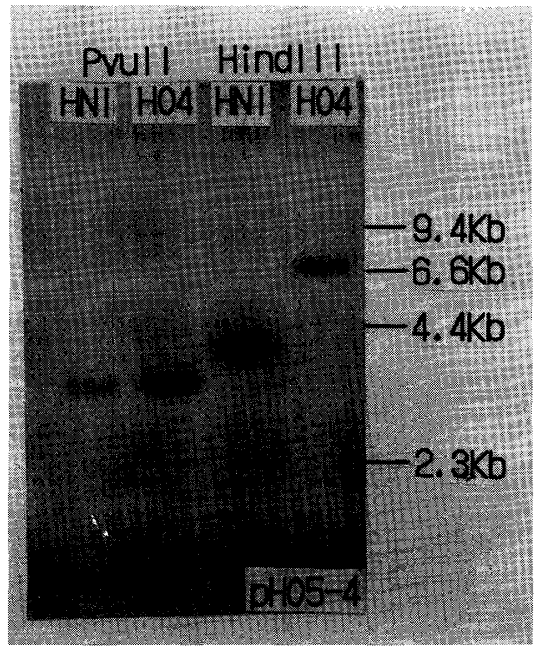

Fig. 1. An example of hybridization.

Table 1. RFLP between HO4C and HNI detected by random genomic clones

Clone No.	*Hin*dIII	*Pvu*II	*Sac*I	*Apa*I	*Eco*RV	*Pst*I	*Eco*RI	*Bgl*II	Var./Enz.
pHO5-2	–	+	n	n	+	–	+	+	4/6
pHO5-3	+	–	–	–	–	–	–	–	1/8
pHO5-4	–	–	+	n	+	–	+	–	3/7
pHO5-5	n	+	n	n	n	+	n	+	3/3
pHO5-6	n	–	+	–	+	–	+	–	3/7
pHO5-8	+	+	+	n	n	–	–	n	3/6
pHO5-9	–	+	+	n	n	–	–	n	2/5
pHO5-11	+	–	n	–	n	n	–	n	1/4
pHO5-14	+	+	–	n	n	–	n	–	2/5
pHO5-16	+	n	n	n	+	n	–	–	2/4

+, RFLP is observed, –, RFLP is not observed, n, not examined.

*, the number of enzymes that can detect polymorphism, among the enzymes examined.

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